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Physiological Consequences of Moisture Deficit Stress in Cotton

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ABSTRACT

Moisture deficits can depress cotton (*Gossypium hirsutum* L.) lint yield in all cotton production regions. However, most cotton physiological drought stress research has been conducted in arid production regions, growth chambers, or greenhouses. The objective of this research was to document the effects of moisture deficit stress on the physiology of cotton grown in the humid southeastern USA. Field studies were conducted under dryland and irrigated conditions from 1998 to 2001 with eight genotypes, including an okra-normal leaftype near-isoline pair and transgenic lines paired with their recurrent parents. Dry matter partitioning, light interception, canopy temperature, leaf water potential, gas exchange, chlorophyll (Chl) fluorescence, and leaf Chl content data were collected. Genotypes responded similarly to both soil moisture regimes. Drought stress reduced overall plant stature with a 35% leaf area index (LAI) reduction, prompting an 8% reduction in solar radiation interception. Dryland leaves had 6% greater CO_2 exchange rates (CER) and 9% higher light-adapted photosystem II (PSII) quantum efficiency than irrigated leaves during the morning. However, as water potential of the dryland plants became more depressed during the afternoon, the CER and light adapted PSII quantum efficiency of the dryland plants became inhibited and was 6 and 10% lower, respectively, than irrigated leaves. A 19% greater Chl content for the dryland leaves contributed to their higher CER during the morning. This polarity of photosynthesis throughout the day for the dryland plants relative to irrigated plants may explain some of the irrigation yield response inconsistencies in the southeastern USA.

ADEQUATE SOIL MOISTURE (provided through timely and adequate irrigation or precipitation events) is essential for successful crop production. Upland cotton is no exception to this requirement. Although wild cotton lines inhabit regions of sparse precipitation (Lee, 1984), irrigation technologies are necessary for the successful commercial production of cotton in arid regions. Irrigation scheduling in desert-like environments such as Arizona and California has been perfected to the point of consistently producing acceptable yield enhancements in cotton production (Radin et al., 1992). However, the yield response of cotton to irrigation in the humid southeastern USA remains inconsistent (Pringle et al., 2003). Understanding the nature of this inconsistent irrigation response requires a more thorough knowledge of cotton's response to varying types of moisture deficit stress. The timing, duration, severity, and speed of development for the moisture deficit stress undoubtedly play pivotal roles in determining how a plant responds to moisture deficit stress.

Although there has been considerable research documenting the growth and physiological response of cotton to moisture deficit stress, most of it has been conducted with pots in artificially controlled growth environments of greenhouses (Jordan, 1970; Radin, 1981; Radin and Ackerson, 1981; Loffroy et al., 1983; Ball et al., 1994) and growth chambers (Genty et al., 1987; Nepomuceno et al., 1998), or has been conducted under field conditions in arid climates (Turner et al., 1986; Puech-Suanzes et al., 1989; Ephrath et al., 1990; Ephrath et al., 1993; Leidi et al., 1993; López et al., 1995; Lacape et al., 1998; Leidi et al., 1999) where moisture deficit stresses are more prevalent and extreme. Field studies under temperate humid conditions have been conducted by McMichael and Hesketh (1982) and Faver et al. (1996). From these studies, we know that moisture deficit stress promotes stunted growth in cotton with reduced leaf area expansion (Turner et al., 1986; Ball et al., 1994; Gerik et al., 1996). Lint yield is generally reduced because of reduced boll production, primarily because of fewer flowers but also because of increased boll abortions when the stress is extreme and when it occurs during reproductive growth (Grimes and Yamada, 1982; McMichael and Hesketh, 1982; Turner et al., 1986; Gerik et al., 1996; Pettigrew, 2004). Leaf photosynthesis is also reduced when plants are grown under moisture deficit conditions because of a combination of stomatal and nonstomatal limitations (McMichael and Hesketh, 1982; Marani et al., 1985; Turner et al., 1986; Genty et al., 1987; Ephrath et al., 1990; Faver et al., 1996). As in most plants, leaf water potential (Ψ_l) is reduced under drought conditions, but cotton has the ability to osmotically adjust and maintain a higher leaf turgor potential (Ψ_t) (Turner et al., 1986; Nepomuceno et al., 1998).

Although these controlled growth environment studies have proven insightful, overall cotton growth and yield is reduced when the root zone volume is constrained by a finite container size (Carmi and Shalhevet, 1983). How applicable these controlled-environment studies are to what the plants would experience and respond to under natural field conditions is not clear. Similarly, the arid environment, where the vast majority of field studies have been conducted, would tend to lead to early, rapid, and extreme moisture deficit stress developing in the

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Abbreviations: CER, CO_2 exchange rate; Chl, chlorophyll; DAP, days after planting; Fv/Fm, dark adapted chlorophyll fluorescence variable to maximal ratio; LAI, leaf area index; q_P, photochemical quenching; PPFD, photosynthetic photon flux density; PSI, photosystem I; PSII, photosystem II; q_{NP}, nonphotochemical quenching; SLW, specific leaf weight.

cotton plants. A later, slower developing, and less severe stress, as would tend to occur in more humid, temperate environments, may modify or delay the physiological alterations in response to moisture deficit stress.

The introduction of transgenic cotton varieties into large-scale production has occurred within the last decade. With the uncertainty of gene insertion position for a given transformation event utilizing current transformation technologies, DNA could be inserted into a chromosomal region containing a gene and thereby disrupt the gene's function. Most of these unfavorable insertions are eliminated through an extensive screening and selection process that culls all lethal mutations and mutations that prove deleterious to one of the major agronomic or quality traits. More subtle alterations could potentially pass through this screen. This possibility, combined with the current cotton yield stagnation and instability problems, has lead to the question of whether transgenic cotton lines are more sensitive to abiotic stresses.

The primary objective of this research was to compare the performance of various physiological traits under irrigated and dryland conditions in a temperate, humid environment for a diverse group of cotton genotypes. A secondary objective was to assess whether transgenic cottons demonstrated enhanced sensitivity to abiotic stress by including two transgenic-recurrent parent pairs among the genotypes grown under the two soil moisture regimes.

MATERIALS AND METHODS

Field studies were conducted during the years 1998 to 2001 on a highly productive Bosket fine sandy loam (fine-loamy, mixed, active, thermic Mollic Hapludalf) near Stoneville, MS. Eight cotton genotypes, 'DPL 20', 'DPL 20B', 'FiberMax 819', 'MD 51 ne normal leaftype', 'MD 51 ne okra leaftype', 'PayMaster H1220', 'PayMaster 1220 BR', and 'STV 474', were grown under both irrigated and dryland conditions. DPL 20B contains the Bt gene that produces an endotoxin lethal to certain lepidopteran insects and DPL 20 is the recurrent parent line to DPL 20B. PayMaster 1220 BR contains both the Bt gene and a glyphosate-resistance gene that conveys resistance to the herbicide glyphosate. PayMaster H1220 is the recurrent parent line to PayMaster 1220 BR. MD 51 ne normal leaftype and MD 51 ne okra leaftype are near isogenic lines varying in leaf shape and were provided by W.R. Meredith Jr. (USDA-ARS, Stoneville, MS). Both MD 51 ne okra leaftype and FiberMax 819 possess the okra leaftype shape which has been reported to convey some drought tolerance characteristics (Karami et al., 1980; Pettigrew et al., 1993; Voloudakis et al., 2002). The genotypes were selected to represent a range of genetic backgrounds.

The experimental design was a randomized complete block with a split-plot arrangement of treatments. Five replicates were used from 1998 through 2000, and four replicates were used in 2001. Two soil moisture treatments (irrigated and dryland) were the main plots and the eight genotypes comprised the subplots. The soil moisture treatment main plots and genotype subplots were randomly assigned each year.

Experimental units or plots consisted of four rows 7.62 m long with a 1-m row spacing and were planted on 23 April 1998, 21 April 1999, and 26 April 2000 and 2001. These plots were initially overseeded and then hand-thinned to the desired population density of approximately 97 000 plants ha^{-1}. The experimental area was subsoiled each fall after cotton stalk destruction. Each year, the experimental area received 112 kg N ha^{-1} in a preplant application. Recommended insect and weed control measures were employed throughout each growing season as needed.

Two soil moisture treatments (irrigated and dryland) were used in the study. In 1998, 1999, and 2000, the irrigated plots received four furrow irrigations for a total of 10.16 cm each year. Only three furrow irrigations totaling 7.62 cm were applied in 2001. Tensiometers were used to monitor soil moisture content at a 30-cm depth, with irrigations triggered when readings reached 40 to 50 centibars. However, this irrigation schedule often had to be adjusted (either accelerated or delayed) to accommodate required insecticide spraying and any resulting restricted reentry interval. To enhance the degree of moisture deficit stress occurring in the dryland treatment, rainfall was prevented from entering the soil by covering the soil surface between rows with black polyethylene film from mid June until after harvest (Pettigrew, 2004).

Dry matter harvests were taken at 69 and 96 days after planting (DAP) in 1998, at 63 and 98 DAP in 1999, at 55 and 88 DAP in 2000, and at 55 and 90 DAP in 2001. The early harvest date roughly corresponded to a harvest date during the early blooming period, while the later harvest date corresponded to a cutout harvest date. Cutout refers to a period of slowing vegetative growth and flowering because of a strong demand for assimilates by the existing boll load. One of the inner two plots rows was designated for use in the dry matter harvests. On each harvest date, the aboveground portions of plants from 0.3 m of row were harvested and separated into leaves, stems and petioles, squares, and blooms and bolls. Leaf area was determined with a LI-3100 leaf area meter (LI-COR, Lincoln, NE[1]), and main-stem nodes were counted. Samples were dried for at least 48 h at 60°C, and dry weights were recorded.

The percentage of photosynthetic photon flux density (PPFD) intercepted by the canopies was determined with a LI 190SB point quantum sensor (LI-COR) positioned above the canopy and a 1-m-long LI 191SB line quantum sensor placed on the ground perpendicular to and centered on the row. Two measurements were taken per plot, and the mean of those two measurements was used for statistical analyses. These measurements were taken under clear skies at 68 and 90 DAP in 1998; 97 DAP in 1999; 68 and 90 DAP in 2000; and 53 and 89 DAP in 2001.

Canopy temperature measurements were taken under clear skies during the afternoon at 75 and 103 DAP in 1998; 70 and 98 DAP in 2000; and 71 and 98 DAP in 2001 by a Telatemp Model AG-42 infrared thermometer (Telatemp Corp., Fullerton, CA). This instrument recorded both canopy surface temperature and the difference between canopy surface temperature and ambient air temperature. Two instantaneous measurements were taken per plot, and the mean of those two measurements were used for statistical analyses.

Water relations data were collected at approximately 1330 h CDT on 96 to 100 DAP in 1999, 89 to 93 DAP in 2000, and 88 to 95 DAP in 2001. Components of leaf water potential (Ψ_l) for the youngest fully expanded leaf per plant (fourth or fifth leaf from the top on the plant) were determined for leaves from three plants per plot with leaf cutter thermocouple psychrometers (JRD Merrill Specialty Equipment, Logan, UT). After rapidly cutting and inserting the leaf disk into the chamber, the samples were equilibrated for 3 h in a 30°C water bath and then the Ψ_l was measured. At least four Ψ_l readings

[1]Trade names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product or service, and the use of the name by USDA implies no approval of the product or service to the exclusion of others that may also be suitable.

were taken on each leaf disk after the period of equilibration. Stable readings from the three psychrometers per plot were averaged together for subsequent statistical analysis. Following Ψ_l determinations, the samples were frozen overnight in a −20°C freezer, then allowed to reequilibrate for another 3 h in the 30° water bath; then the leaf osmotic potential (Ψ_π) was determined. Leaf turgor (Ψ_t) was estimated as the difference between Ψ_l and Ψ_π.

Leaf CER and other gas exchange parameters were measured on the youngest fully expanded, disease-free, fully sunlit leaves in each plot with a LI-6200 portable photosynthesis system (LI-COR) with a 1-L chamber. All measurements were taken between 0900 and 1200 CDT with individual leaves oriented perpendicular to the sun. The PPFD reaching the adaxial leaf surfaces were ≥1600 μmol m^{-2} s^{-1} on all measurements. Measurements were taken on two leaves per plot with the average of the two leaves used for all statistical analysis.

Chlorophyll variable fluorescence/maximal fluorescence (Fv/Fm) ratios were taken on the same two leaves per plots as used in the CER measurements. In 1998, a CF-1000 Fluorescence Measurement System (P.K. Morgan, Inc., Andover, MA) was used to make the measurements, while in 1999 through 2001, a Hansatech Fluorescence Monitoring System (Hansatech Instruments Ltd., Norfolk, UK). Leaves were allowed to dark adapt for at least 15 min after the CER measurements and before the Chl fluorescence measurements. F_o was determined with a weak, modulated amber light. F_m was determined after a 0.8-s pulse of strong white light (>4000 μmol m^{-2} s^{-1} PPFD). In 1999 through 2001, after the dark adapted Chl Fv/Fm measurement, the leaves were exposed and acclimated to 650 μmol m^{-2} s^{-1} PPFD at the leaf surface for 90 sec. After this period of acclimation, light adapted Chl fluorescence yields (F_s, steady state fluorescence yield; and F'_m, light adapted fluorescence maximum) were measured at 650 μmol m^{-2} s^{-1} PPFD. From these light adapted values, the quantum efficiency of photosystem II (ϕ_{PSII}) and electron transport rate were derived. Coupling these light adapted measurements with the dark adapted Fv/Fm measurements allows for determination of the extent of photochemical (qP) and nonphotochemical (qNP) quenching at this level of light adaptation (Schreiber et al., 1986). The average of the two measurements per plot were used for the statistical analyses.

To document alterations in CER and Chl fluorescence behavior at different times during the day, measurements were taken first before solar noon and then again after solar noon on the same day in 2001. The two leaves measured before solar noon were tagged and then measured again after solar noon on the same day. As with the other CER measurements, the PPFD reaching the adaxial leaf surfaces were ≥1600 μmol m^{-2} s^{-1} on all measurements.

Upon completion of the CER and Chl fluorescence, the leaves were collected for specific leaf weight (SLW) and leaf Chl content determinations. One of the leaves had its leaf area determined and dry weight measured (48 h at 60°C) to calculate SLW. Leaf disks were collected from the second leaf for Chl content assays. Chlorophyll was extracted over a 24-h period in darkness at 30°C from two 0.4 cm^2 leaf disks per leaf in 950 mL L^{-1} ethanol. The Chl concentration of the extract was then spectrophotometrically determined according to the methods of Holden (1976).

Statistical analyses were performed by ANOVA (PROC MIXED, SAS Institute, 1996). For traits where year interacted with treatments, or genotypes and environmental effects associated with year were identified, the results were presented by year. When the treatment or genotype differences for a trait were consistent across years, then treatment or genotype means were averaged across years and the year interactions with treatment or genotype were considered a random source of error. When statistically significant interactions were not detected, treatment means were averaged across genotypes and genotype means were averaged across treatments. Means were separated by a protected LSD at the $P \leq 0.05$ level.

RESULTS

Climatic Conditions

Year-to-year variability among climatic factors ensured four distinct environments for testing the study objectives. While the weather data and soil moisture data for this site have been previously reported (Pettigrew, 2004), a brief synopsis is appropriate for this report. Approximately 22.9 cm of rain was received during July and August (the period of flowering and boll set) in 1998 and 2001 compared with an average of 2.4 cm of rain in 1999 and 2000. The extra precipitation in 2001 was accompanied by a reduction in the solar radiation and by cooler temperatures. Because of the reduced precipitation received in 1999 and 2000, a 24% greater soil moisture deficit developed in the dryland plots during those years compared with 1998 and 2001.

Soil Moisture Effects

The most obvious soil moisture deficit response across time is a reduction in plant stature. Because soil moisture treatments did not interact with genotypes or years, treatment means were averaged across genotypes and years. By the early bloom dry matter harvest, the moisture deficit stress in the dryland plots had not become severe enough to impact any of the growth parameters measured (Table 1). None of the traits differed signifi-

Table 1. Cotton dry matter partitioning and canopy photosynthetic photon flux density (PPFD) interception as affected by two soil moisture regimes (dryland and irrigated) both early and late in the blooming period, averaged across eight genotypes and the years 1998 to 2001.

Growth stage	Moisture treatment	Height	Main stem nodes	Height-to-node ratio	Leaf area index	Specific leaf weight	Vegetative weight	Reproductive weight	Harvest index†	PPFD interception
		cm	nodes $plant^{-1}$	cm $node^{-1}$		g m^{-2}				%
Early bloom	dryland	58	14.7	3.92	1.48	57.8	160.0	10.9	0.054	64.3
	irrigated	60	15.1	3.99	1.72	57.3	183.9	10.6	0.045	62.9
	LSD (0.05)	11	0.4	0.62	0.59	3.6	54.8	3.6	0.025	11.1
	P > *F*	0.49	0.09	0.75	0.29	0.69	0.25	0.90	0.35	0.63
Late bloom	dryland	92	20.5	4.51	2.59	57.9	331.7	200.4	0.371	83.5
	irrigated	110	23.1	4.77	3.99	51.6	486.1	201.2	0.286	91.0
	LSD (0.05)	10	0.5	0.51	0.31	3.6	63.9	17.9	0.023	4.2
	P > *F*	0.01	0.01	0.21	0.01	0.01	0.01	0.93	0.01	0.01

† Harvest index = reproductive dry weight/total aboveground dry weight.

Table 2. Cotton leaf water potential (ψ_l), leaf osmotic potential (ψ_π), and leaf turgor potential (ψ_t) measured in the afternoon as affected by two soil moisture regimes (dryland and irrigated) averaged across eight genotypes and the years 1999 to 2001.

Moisture treatment	ψ_l	ψ_π	ψ_t
	MPa		
Dryland	−2.36	−2.50	0.14
Irrigated	−1.74	−1.93	0.20
LSD (0.05)	0.38	0.27	0.13
$P > F$	0.01	0.01	0.21

cantly among soil moisture treatments during this early bloom harvest period. However, by the late bloom, a severe-enough moisture deficit stress had developed to impact most of the growth parameters. Plants in the dryland plots were 16% shorter than the irrigated plants. The taller irrigated plants were caused by the production of 11% more main stem nodes compared with the dryland plants rather than increased internode lengths, because the height-to-node ratio did not differ between soil moisture treatments. These shorter dryland plants also produced 35% less LAI, and thereby reduced overall vegetative growth by 32% compared with the irrigated plants. Although the dryland plants had reduced LAI, the 12% greater SLW of these plants indicate that the leaves may have been thicker or denser than leaves of the irrigated plants. Reproductive growth had not been altered by soil moisture treatment at this stage of growth. However, the similar reproductive weights coupled with reduced vegetative growth of the dryland plants led to a 30% greater harvest index for the dryland plants at this growth stage.

The shorter stature and reduced LAI of the dryland plants meant that those canopies intercepted less solar radiation than canopies of irrigated plants (Table 1). In fact, the canopy PPFD interception differences between soil moisture treatments closely matched the LAI treatment differences. Similar to the LAI data, no soil moisture treatment differences were detected during the early bloom canopy PPFD interception measurements, but by late bloom, the dryland plants were intercepting 8% less PPFD than the irrigated plants.

Water relations of the leaves as measured during the late bloom period were altered by the soil moisture treatments. Afternoon leaf water potentials were 36% more negative in leaves of the dryland plants compared with leaves of the irrigated plants (Table 2). Because the moisture deficit stress was slow to develop, the leaves of the dryland plants were able to osmotically adjust to the developing moisture deficit stress. This osmotic adjustment meant that while the dryland plants had a 30% more negative leaf osmotic potential, they were able to maintain similar leaf turgor as leaves from the irrigated plants. This reduced osmotic potential caused the reduced leaf water potential of the dryland plants at this time, not a loss of leaf turgor. Similar osmotic adjustment has been reported for cotton by Turner et al. (1986) and Nepomuceno et al. (1998).

Canopy surface temperature measured at early bloom and late bloom help document the timing and severity of the moisture deficit development. At early bloom, similar canopy temperatures were detected for the two soil moisture treatments (dryland = 32.1°C, irrigated = 31.8°C), indicative that little moisture deficit stress had developed by this stage. The dryland plants had a significantly higher canopy temperature by late bloom (35.2°C dryland vs. 30.7°C irrigated), and the canopy-to-ambient air temperature difference at that time was significantly lower in the dryland plants (−4.1°C) compared with the irrigated plants (−8.9°C), indicative of less transpirational cooling of leaves for the dryland plants. The higher canopy temperatures coupled with the lower leaf water potentials of the dryland plants during the late bloom stage demonstrate the level of moisture deficit stress in the dryland plots relative to the irrigated.

Averaged across years, morning CER measurements were 6% greater for leaves from the dryland plants compared with irrigated leaves (Table 3). This result is in contrast to the many reports of lower leaf photosynthesis under moisture deficit conditions (McMichael and Hesketh, 1982; Marani et al., 1985; Turner et al., 1986; Genty et al., 1987; Ephrath et al., 1990; Faver et al., 1996). This higher photosynthesis seen in dryland leaves was not accompanied by higher stomatal conductance, and therefore produced a tendency for lower internal CO_2 concentrations and for the water use efficiency to be higher with dryland plants. While the maximum quantum efficiency of PSII photochemistry, as measured by the dark adapted Fv/Fm, did not vary between soil moisture treatments, the light adapted PSII quantum efficiency (adapted at 650 μmol m^{-2} s^{-1} PPFD) was 9% greater in the dryland leaves compared with the irrigated leaves and allowed for a 9% greater electron transport rate in the dryland leaves (Table 4). While qP was similar between soil moisture treatments, the nonphotochemical quenching was 6% lower in leaves from the dryland plants. Photochemical quenching is caused by the oxidation of the primary electron acceptor (Q_A) of PSII; in the light this is caused by electron transport through photosystem I (PSI). Nonphotochemical quenching can be caused by (i) the intrathylakoid acidification during light-driven proton translocation across the membrane; (ii) increased distribution of excitation energy to weakly fluorescent PSI

Table 3. Various cotton leaf gas exchange parameters measured during the morning as affected by two soil moisture regimes (dryland and irrigated) averaged across eight genotypes and the years 1999 to 2001.

Moisture treatment	CO_2 exchange rate	Internal CO_2 concentration	CO_2 stomatal conductance	Water use efficiency
	μmol m^{-2} s^{-1}	μL L^{-1}	mol m^{-2} s^{-1}	mmol CO_2 mol H_2O^{-1}
Dryland	33.3	286	0.76	2.06
Irrigated	31.5	290	0.78	1.92
LSD (0.05)	1.5	5	0.05	0.17
$P > F$	0.03	0.07	0.31	0.08

Table 4. The response of morning cotton leaf dark adapted variable to maximal chlorophyll (Chl) fluorescence ratio (Fv/Fm) and various light adapted Chl fluorescence parameters to two soil moisture regimes (dryland and irrigated), averaged across eight genotypes and years. Upon completion of the dark adapted measurements, leaves were light adapted at 650 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and then the light adapted Chl fluorescence measured at 650 μmol m^{-2} s^{-1} PPFD.

Moisture treatment	Dark adapted Fv/Fm†	Photosystem II quantum efficiency (Φ_{II})‡	Electron transport rate	Quenching coefficients	
				Photochemical	Nonphotochemical
			μmol m^{-2} s^{-1}		
Dryland	0.729	0.459	125	1.205	0.760
Irrigated	0.705	0.420	115	1.322	0.810
LSD (0.05)	0.051	0.032	9	2.619	0.047
$P > F$	0.22	0.02	0.02	0.67	0.04

† Dark adapted Fv/Fm measurements were averaged across the years 1998 to 2001.
‡ Φ_{II}, electron transport rate, and the quenching coefficients were averaged across 2000 and 2001.

at the expense of PSII excitation, regulated by phosphorylation of LHC II; and (iii) photoinhibition of photosynthesis (Krause and Weis, 1991). These fluorescent quenching results contrast with the findings of Genty et al. (1987), who reported that qP was depressed by drought stress but that nonphotochemical quenching was relatively unaffected for pot-grown cotton plants.

To address the discrepancy between our higher morning CER with dryland plants compared with the lower CER reported in the literature for drought-stressed plants, gas exchange and Chl fluorescence measurements were taken both before and after solar noon on the same leaves at similar light intensities in 2001. There was a morning vs. afternoon effect on the response of CER, stomatal conductance, light adapted PSII quantum efficiency, and photosynthetic electron transport rates to the two soil moisture regimes (Table 5). Similar to the morning CER and stomatal conductance measurements averaged across years, in 2001 dryland plants had a 6% greater morning CER but similar stomatal conductances compared with irrigated plants. By the afternoon, the CER response had been reversed with leaves from the dryland plants having a 6% lower CER than the irrigated plants. Accompanying this CER decline was a 24% reduction in stomatal conductance for the dryland plants relative to the irrigated plants. While the maximum quantum efficiency of PSII photochemistry did not vary in response to the soil moisture treatments during either the morning and afternoon measurement periods, the light adapted PSII quantum efficiency of the dryland plant leaves was 10% lower in the afternoon compared with the irrigated, with a corresponding 10% reduction in photosynthetic electron transport rate.

Three out of four years, the leaves from the dryland plants averaged 19% greater Chl content than leaves from the irrigated plants (Table 6). This greater Chl content was accompanied by reduced leaf size in the dryland plants during three out of four years. During the only year when there was a statistical difference between soil moisture treatment for SLW, the dryland plants had a greater individual leaf SLW than the irrigated plants, which matches the greater overall SLW for the dryland plants during the late bloom dry matter harvest (Table 1). This higher leaf Chl content of the dryland leaves may contribute to the higher morning CER for the dryland plants. By afternoon, the reduced leaf water potential overwhelmed any advantage the higher Chl content gave the dryland plants and promoted the lower CER at that time of day relative to the irrigated plants (Faver et al., 1996).

Genotypic Effects

Averaged across the soil moisture treatments, genotypic variation was detected for morning CER and many of the components of the photosynthetic process (Table 7). Similar to previous research, the two okra leaftype varieties exhibited 30% higher CER on average than any of the normal leaftype varieties (Pettigrew et al., 1993). Although previous research indicated that okra leaftype genotypes also had lower stomatal conductances (Pettigrew et al., 1993), that was not found to be the case with the okra leaftype lines used in this study. The use of different genetic backgrounds containing the okra leaftype trait between the two studies is probably responsible for these contrasting stomatal conductance

Table 5. Cotton leaf gas exchange and chlorophyll fluorescence parameters measured on the same leaves in both the morning and afternoon as affected by two soil moisture regimes (dryland and irrigated) averaged across eight genotypes.

Time of day	Moisture treatment	CO_2 exchange rate	CO_2 stomatal conductance	Dark adapted Fv/Fm†	Photosystem II quantum efficiency (Φ_{II})‡	Electron transport rate
		μmol m^{-2} s^{-1}	mol m^{-2} s^{-1}			μmol m^{-2} s^{-1}
Morning	Dryland	35.3	0.73	0.781	0.467	127
	Irrigated	33.2	0.71	0.789	0.438	120
	LSD (0.05)	1.8	0.07	0.028	0.030	8
	$P > F$	0.02	0.49	0.52	0.06	0.06
Afternoon	Dryland	29.7	0.53	0.722	0.394	108
	Irrigated	31.6	0.70	0.746	0.436	119
	LSD (0.05)	2.0	0.07	0.030	0.034	9
	$P > F$	0.06	0.01	0.10	0.02	0.02

† Dark adapted chlorophyll fluorescence variable to maximal ratio.
‡ Φ_{II} and electron transport rate were light acclimated and measured at 650 μmol m^{-2} s^{-1} photosynthetic photon flux density.

Table 6. Cotton leaf chlorophyll (Chl) content, leaf area, and specific leaf weight (SLW) as affected by two soil moisture regimes (dryland and irrigated) averaged across eight genotypes for the years 1998 to 2001.

Year	Moisture treatment	Chl content	Leaf area	SLW
		mg m^{-2}	cm^2	g m^{-2}
1998	dryland	360	99	62.4
	irrigated	293	114	66.3
	LSD (0.05)	41	13	4.5
	$P > F$	0.01	0.04	0.08
1999	dryland	417	99	74.3
	irrigated	418	120	75.1
	LSD (0.05)	45	5	5.3
	$P > F$	0.97	0.01	0.75
2000	dryland	480	106	80.5
	irrigated	411	104	70.5
	LSD (0.05)	37	9	5
	$P > F$	0.01	0.62	0.01
2001	dryland	465	80	77.5
	irrigated	397	97	71.1
	LSD (0.05)	44	5	7.0
	$P > F$	0.01	0.01	0.06

results. The dark adapted Fv/Fm was no different for the okra leaftype genotypes compared with the normal leaftype genotypes; however, the okra leaftype lines had a 14% greater light adapted PSII quantum efficiency and 14% greater photosynthetic electron transport rate compared with the normal leaftype genotypes. Nonphotochemical quenching was also 11% lower in the okra leaftype genotypes relative to the normal leaftype genotypes.

The okra leaf trait reduced the individual leaf area 37% relative to the comparable normal leaftype leaves (Table 8). MD 51 ne okra leaftype had 16% greater leaf Chl content compared with MD 51 ne normal leaftype, its normal leaftype near-isogenic pair. While this result is similar to the greater leaf Chl content for okra leaftype lines reported previously (Pettigrew et al., 1993), FiberMax 819 did not exhibit significantly greater leaf Chl content than many of the normal leaftype genotypes. This contrasting result is probably because of the comparison involving different genetic backgrounds in addition to different leaftypes. The two okra leaftype lines used in this study also did not exhibit higher SLW relative to the normal leaftype genotypes, which is in contrast to greater SLW for the okra leaftype trait in a different genetic background from the previous study (Pettigrew et al., 1993).

Table 8. Genotypic variation in cotton leaf chlorophyll (Chl) content, leaf area, and specific leaf weight (SLW) averaged across two soil moisture regimes (dryland and irrigated) and the years 1998 to 2001.

Genotype	Chl content	Leaf area	SLW
	mg m^{-2}	cm^2	g m^{-2}
DPL 20	379	110	75.7
DPL 20B	402	117	74.3
FiberMax 819	420	80	70.4
MD 51 ne normal	389	109	71.5
MD 51 ne okra	451	63	70.1
PayMaster 1220 BR	397	116	71.2
PayMaster H1220	377	114	71.7
Stoneville 474	426	111	72.8
LSD (0.05)	24	11	3.7
$P > F$	0.01	0.01	0.04

DISCUSSION

The moisture deficit response for many traits measured in this study are similar to moisture deficit responses for cotton grown in arid field conditions or in artificial environments of growth chambers and greenhouses. The reduced plant stature and LAI under moisture deficit stress (Table 1) are similar to that reported by Jordan (1970), McMichael and Hesketh (1982), Turner et al. (1986), Ball et al. (1994), and Gerik et al. (1996). Reduction in leaf area expansion under moisture deficit conditions (McMichael and Hesketh, 1982; Turner et al., 1986; Ball et al., 1994) undoubtedly lead to the smaller leaf sizes of the youngest fully expanded leaves used in the photosynthesis measurements for the dryland plants. The higher SLW of the dryland plants is similar to that reported by Wilson et al. (1987). This combination of reduced plant stature with fewer and smaller leaves lead to the dryland canopy intercepting less PPFD during the late bloom period, when the moisture deficit was at full development, than the irrigated canopy (Table 1).

Although many previous studies have documented reduced photosynthesis associated with moisture deficit stress (McMichael and Hesketh, 1982; Marani et al., 1985; Turner et al., 1986; Genty et al., 1987; Ephrath et al., 1990; Faver et al., 1996) and the further depression of

Table 7. Cotton genotypic variation in morning leaf gas exchange parameters, dark adapted variable to maximal chlorophyll (Chl) fluorescence ratio (Fv/Fm), and various other Chl fluorescence parameters measured at 650 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) after acclimation at 650 μmol m^{-2} s^{-1} PPFD, averaged across two soil moisture regimes (dryland and irrigated) and years.

Genotype	CO_2 exchange rate†	Internal CO_2 concentration	CO_2 stomatal conductance	Dark adapted Fv/Fm	Photosystem II quantum efficiency (Φ_{II})‡	Electron transport rate	Quenching coefficients	
							Photochemical	Nonphotochemical
	μmol m^{-2} s^{-1}	μL L^{-1}	mol m^{-2} s^{-1}			μmol m^{-2} s^{-1}		
DPL 20	29.8	286	0.73	0.715	0.420	115	1.298	0.808
DPL 20B	29.8	288	0.75	0.725	0.429	117	1.411	0.807
FiberMax 819	37.4	290	0.77	0.714	0.473	129	1.080	0.735
MD 51 ne normal	30.1	289	0.77	0.687	0.427	117	1.372	0.784
MD 51 ne okra	41.1	289	0.80	0.723	0.497	136	0.961	0.700
PayMaster 1220 BR	30.5	288	0.78	0.729	0.420	115	1.224	0.800
PayMaster H1220	30.3	289	0.80	0.716	0.419	114	1.409	0.824
Stoneville 474	30.3	285	0.73	0.727	0.429	117	1.356	0.823
LSD (0.05)	1.2	3	0.05	0.021	0.035	10	0.444	0.065
$P > F$	0.01	0.03	0.03	0.01	0.01	0.01	0.33	0.01

† Gas exchange parameters and dark adapted Fv/Fm measurements were averaged across the years 1998 to 2001.
‡ Φ_{II}, electron transport rate, and the quenching coefficients were averaged across 2000 and 2001.

photosynthesis as the day progressed into the afternoon (Turner et al., 1986; Puech-Suanzes et al., 1989; Ephrath et al., 1990; Ephrath et al., 1993), this study conducted under humid environmental conditions is the first to document a moisture-deficit stress-induced elevated photosynthesis in the morning before plummeting to lower photosynthetic rates in the afternoon compared with irrigated plants (Table 5). This study also documented that while the dark adapted Chl, fluorescence Fv/Fm (maximum PSII quantum efficiency) was not altered by the soil moisture treatments, the light adapted PSII quantum efficiency and photosynthetic electron transport of the dryland leaves were greater during the morning before falling below levels exhibited by the irrigated leaves in the afternoon. Data from this research indicate that both stomatal and nonstomatal factors contributed to this afternoon photosynthetic decline in the dryland leaves.

Smaller leaves with occasionally greater SLW for the dryland plants (Table 6) leads to speculation of a higher concentration of photosynthetic apparatus per unit leaf area for the dryland plants. This speculation is reinforced by the 19% greater Chl content of the dryland plant leaves. These deep Mississippi Delta soils allowed the moisture deficit stress to be slow-developing for the dryland plants. The capacity of these soils to recharge the plant's hydraulic network overnight also allowed the dryland plants to take advantage of this potentially higher photosynthetic apparatus density per unit leaf area, and photosynthesize at a higher rate during the morning before the decreasing leaf water potential became the dominant influence and began shutting down components of the photosynthetic process. Soils under more severe moisture deficit conditions, as in arid environments, or without as large a recharge capacity, may not sufficiently recharge the plant to allow for the higher morning photosynthetic potential to express itself.

The primary genotypic variation seen in the physiological traits measurements was the elevated CER observed with the two okra leaftype lines used in this study compared with the normal leaftype lines. These results were similar to the previously reported higher okra leaftype CER rates in a different genetic background (Pettigrew et al., 1993). The 14% greater light adapted PSII quantum efficiency and electron transport rate, coupled with the 11% reduction in nonphotochemical quenching is new information regarding genotypic variation in photosynthetic components and further explains the higher photosynthetic rates per unit leaf area observed with okra leaftype genotypes. Few, if any, differences were detected for any of the physiological traits measured between the transgenic lines and their conventional recurrent parent lines. The transformation events that introduced the Bt and glyphosate resistance genes did not disrupt the photosynthetic response to developing soil moisture deficits. It appears that the transgenic genotypes did not respond any differently to moisture stress than did their conventional recurrent parent lines.

CONCLUSIONS

In conclusion, soil moisture deficit stress in the humid environment of the mid-southern USA reduces cotton plant stature and LAI, promoting an accompanying reduction in the solar radiation intercepted by the canopy. Although there is less leaf area intercepting a reduced portion of the incoming solar radiation, leaves from dryland plants have the potential for elevated photosynthetic performance during the morning hours when the hydraulic status of the plants is still at an acceptable level. As the day progresses and evapotranspirational demand exceeds the moisture recharging capacity of the plant and soil, the hydraulic status of the plant deteriorates to the point of causing the photosynthetic reduction seen during the afternoon relative to irrigated plants. This polarity of photosynthetic performance throughout the day of the dryland plants relative to irrigated plants may help to explain some of the irrigation inconsistencies for lint yield production in the humid southeastern USA. The consistency of the moisture deficit response across genotypes indicates that the current transgenic varieties are not more susceptible to soil moisture deficit than the current conventional varieties.

REFERENCES

Ball, R.A., D.M. Oosterhuis, and A. Mauromoustakos. 1994. Growth dynamics of the cotton plant during water-deficit stress. Agron. J. 86:788–795.

Carmi, A., and J. Shalhevet. 1983. Root effects on cotton growth and yield. Crop Sci. 23:875–878.

Ephrath, J.E., A. Marani, and B.A. Bravdo. 1990. Effects of moisture stress on stomatal resistance and photosynthetic rate in cotton (*Gossypium hirsutum*) I. Controlled levels of stress. Field Crops Res. 23:117–131.

Ephrath, J.E., A. Marani, and B.A. Bravdo. 1993. Photosynthetic rate, stomatal resistance and leaf water potential in cotton (*Gossypium hirsutum* L.) as affected by soil moisture and irradiance. Photosynthetica 29:63–71.

Faver, K.L., T.J. Gerik, P.M. Thaxton, and K.M. El-Zik. 1996. Late season water stress in cotton: II. Leaf gas exchange and assimilation capacity. Crop Sci. 36:922–928.

Genty, B., J.M. Briantais, and J.B. Vieira Da Silva. 1987. Effects on drought on primary photosynthetic processes of cotton leaves. Plant Physiol. 83:360–364.

Gerik, T.J., K.L. Faver, P.M. Thaxton, and K.M. El-Zik. 1996. Late season water stress in cotton: I. Plant growth, water use, and yield. Crop Sci. 36:914–921.

Grimes, D.W., and H. Yamada. 1982. Relation of cotton growth and yield to minimum leaf water potential. Crop Sci. 22:134–139.

Holden, M. 1976. Chlorophylls. p. 1–37. *In* T.W. Goodwin (ed.) Chemistry and biochemistry of plant pigments. Academic Press, New York.

Jordan, W.R. 1970. Growth of cotton seedlings in relation to maximum daily plant-water potential. Agron. J. 62:699–701.

Karami, E., D.R. Krieg, and J.E. Quisenberry. 1980. Water relations and carbon-14 assimilation of cotton with different leaf morphology. Crop Sci. 20:421–426.

Krause, G.H., and E. Weis. 1991. Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 313–349.

Lacape, M.J., J. Wery, and D.J.M. Annerose. 1998. Relationships between plant and soil water status in five field-grown cotton (*Gossypium hirsutum* L.) cultivars. Field Crops Res. 57:29–43.

Lee, J.A. 1984. Cotton as a world crop. p. 1–25. *In* R.J. Kohel and C.F. Lewis (ed.) Cotton. Agron. Monogr. 24. ASA, CSSA, and SSSA, Madision, WI.

Leidi, E.O., M. López, J. Gorham, and J.C. Gutiérrez. 1999. Variation in carbon isotope discrimination and other traits related to drought tolerance in upland cotton cultivars under dryland conditions. Field Crops Res. 61:109–123.

Leidi, E.O., J.M. López, M. López, and J.C. Gutiérrez. 1993. Searching for tolerance to water stress in cotton genotypes: Photosynthesis, stomatal conductance and transpiration. Photosynthetica 28:383–390.

Loffroy, O., C. Hubac, and J.B. Vieira da Silva. 1983. Effect of temperature on drought resistance and growth of cotton plants. Physiol. Plant. 59:297–301.

López, M., J.C. Gutiérrez, and E.O. Leidi. 1995. Selection and characterization of cotton cultivars for dryland production in the southwest of Spain. Eur. J. Agron. 4:119–126.

Marani, A., D.N. Baker, V.R. Reddy, and J.M. McKinion. 1985. Effect of water stress on canopy senescence and carbon exchange rates in cotton. Crop Sci. 25:798–802.

McMichael, B.L., and J.D. Hesketh. 1982. Field investigations of the response of cotton to water deficits. Field Crops Res. 5:319–333.

Nepomuceno, A.L., D.M. Oosterhuis, and J.M. Stewart. 1998. Physiological response of cotton leaves and roots to water deficit induced by polyethylene glycol. Environ. Exp. Bot. 40:29–41.

Pettigrew, W.T. 2004. Moisture deficit effects on cotton lint yield, yield components, and boll distribution. Agron. J. 96:377–383.

Pettigrew, W.T., J.J. Heitholt, and K.C. Vaughn. 1993. Gas exchange and comparative anatomy among cotton leaf-type isolines. Crop Sci. 33:1295–1299.

Pringle, H.C., III, and S.W. Martin. 2003. Cotton yield response and economic implications to in-row subsoil tillage and sprinkler irrigation. J. Cotton Sci. 7:185–193.

Puech-Suanzes, I., T.C. Hsiao, E. Fereres, and D.W. Henderson. 1989. Water-stress effects on the carbon exchange rates of three upland cotton (*Gossypium hirsutum* L.) cultivars in the field. Field Crops Res. 21:239–255.

Radin, J.W. 1981. Water relations of cotton plants under nitrogen deficiency. IV. Leaf senescence during drought and its relation to stomatal closure. Physiol. Plant. 51:145–149.

Radin, J.W., and R.C. Ackerson. 1981. Water relations of cotton plants under nitrogen deficiency. III. Stomatal conductance, photosynthesis, and abscisic acid accumulation. Plant Physiol. 67:115–119.

Radin, J.W., L.L. Reaves, J.R. Mauney, and O.F. French. 1992. Yield enhancement in cotton by frequent irrigations during fruiting. Agron. J. 84:551–557.

SAS Institute. 1996. SAS system for mixed models. SAS Institute, Cary, NC.

Schreiber, U., W. Schliwa, and U. Bilger. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorimeter. Photosynth. Res. 10:51–62.

Turner, N.C., A.B. Hearn, J.E. Begg, and G.A. Constable. 1986. Cotton (*Gossypium hirsutum* L.): Physiological and morphological responses to water deficits and their relationship to yield. Field Crops Res. 14:153–170.

Voloudakis, A.E., S.A. Kosmas, S. Tsakas, E. Eliopoulos, M. Loukas, and K. Kosmidou. 2002. Expression of selected drought-related genes and physiological response of Greek cotton varieties. Funct. Plant Biol. 29:1237–1245.

Wilson, R.F., J.J. Burke, and J.E. Quisenberry. 1987. Plant morphological and biochemical responses to field water deficits. II. Responses of leaf glycerolipid composition in cotton. Plant Physiol. 84:251–254.